BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Sep; 16 (5): 412—414

Effects of tetrandrine and chlorpromazine on synthesis of collagen and hyaluronic acid in cultured human lung fibroblasts

LIU Hai-Lin[1], LU Han-Ming, LI Ding-Guo, JIANG Zu-Ming, QI Feng
(Department of Internal Medicine, Xin-Hua Hospital, Shanghai Second Medical University, Shanghai 200092, China)

AIM: To study the effects of tetrandrine (Tet) and chlorpromazine (Chl) on synthesis of collagen and hyaluronic acid (HA) in cultured human lung fibroblasts (HLF). **METHODS:** The synthesis of collagen and HA was assessed by measuring the incorporation of [^{3}H]proline and radioimmunoassay. **RESULTS:** Both Tet (5—80 μmol L^{-1}) and Chl (10—40 μmol L^{-1}) diminished the collagen synthesis in a concentration-dependent manner. The suppression was aggravated at 36—48 h. The HA content in the supernatant of culture also decreased gradually with the increasing dosage of Tet or Chl after 24-h exposure. There was no obvious toxic effect of Tet on HLF cells at 5—20 μmol L^{-1}. **CONCLUSION:** Tet 5—20 μmol L^{-1} decreased the production of collagen and HA without obvious toxity on HLF, suggesting that Tet could be a hopeful anti-fibrosis drug.

KEY WORDS tetrandrine; chlorpromazine; collagen; hyaluronic acid; fibroblasts; cultured cells

Ca^{2+} indirectly affects intracelluar processes by combining with its receptor protein, calmodulin (CaM), to form the second messenger-complex[1,2]. Tetrandrine (Tet), an alkaloid from *Stephania tetrandra* S Moore, prevents Ca^{2+} influx from extracellular by blocking calcium channels of the cell membrane[3]. Tet possesses an inhibitory effect on silica-induced lung fibrogenesis[4]. So far, few reports dealt with the effects of CaM antagonist on fibroblasts. This paper was to study the effects of Tet and chlorpromazine (Chl) on synthesis of collagen and hyaluronic acid (HA) in cultured human lung fibroblasts (HLF) and explore whether Ca^{2+} antagonist can be used to treat organ fibrosis.

MATERIALS AND METHODS

Cell HLF was provided by Shanghai Institute of Cell Biology, Chinese Academy of Sciences.

Reagents Tet was purchased from Jinghua Pharmaceutical Factory, Zhejiang. Chl was the product of Shanghai-Tianfeng Pharmaceutical Factory (Lot 910901). [^{3}H]Proline was purchased from Chinese Academy of Atomic Energy Science (radioactivity 296 TBq mol^{-1}). RPMI-1640 medium (Gibco). Scintillation liquid was xylene containing 0.5 % PPO and 0.005 % POPOP. Other reagents are all of AR. HA kit was provided by Biochemical Technical Center, Marine Medical Research Institute, Shanghai.

Measurement of collagen synthesis HLF was suspended in RPMI-1640 medium (1.2×10^8 cells L^{-1}) supplemented with 10 % calf serum containing penicillin 100 kU L^{-1} and streptomycin 100 μg L^{-1}, and were plated in 48-well cell culture cluster dishes (1.0 mL/well). Cells were grown in a humidified 5 % CO_2+95 % air at 37 C. After 24-h incubation, Tet and Chl were added separately, and for the untreated controls, an equal amount of drug-solvent (RPMI-1640 containing HCl 0.02 mol L^{-1}, or 0.9 % NaCl) were added. The collagen synthesis was assessed by the incorporation of [^{3}H]proline. The final concentration of [^{3}H]proline was 296 MBq L^{-1}. The cells

[1] Now in *Department of Internal Medicine, the Ninth People's Hospital, Shanghai Second Medical University, Shanghai 200011, China.*

Received 1993-07-07 Accepted 1994-12-26

were exposed to [^{3}H]-proline in the culture for 24 h. At the termination of culture, the cells were treated with 0.25 % trypsin and harvested onto glass fiber filters. They were fixed with trichloroacetic acid 0.6 mol L^{-1} after rinsing with 0.9 % NaCl, and dehydrated and decolorized with ethanol, and stoved at 80 C. The radioactivities (dpm) were counted in a liquid scintillation counter (YSJ 80). Data were expressed as $\bar{x}\pm s$ ($n=3$ wells).

Measurement of HA contents The supernatant of the culture was collected before harvesting cells. Duplicate determinations were done and the data represent the mean of HA concentration in a gamma-radioimmuno-counter (FMJ 182).

Statistical significance was assessed by ANOVA.

RESULTS

Cell morphology under light microscope HLF showed no obvious change in the groups of Tet (5—20 μmol L^{-1}). After 2-h exposure to Tet 40 μmol L^{-1}, cells shrank to become spindle-shaped, and appeared to be slightly sparse, but the cells became normal again after 12 h. However, the cells became round after 1-h exposure to Tet 80 μmol L^{-1}. After 1-h exposure to Chl cells became round, even dropped in 40 μmol L^{-1}. Cells shrank and appeared to be sparse, and part of them became round in 20 μmol L^{-1}. Cells showed no obvious change in 10 μmol L^{-1}. After 12-h exposure to Chl, all cells became round in 20 μmol L^{-1}. Part of cells became round in 10 μmol L^{-1}.

Collagen synthesis of HLF Both Tet and Chl diminished the collagen synthesis in a concentration-dependent manner. The suppression was aggravated at 36—48 h (Tab 1).

HA synthesis of HLF The HA content in the supernatant of culture decreased gradually with the increasing dosage of Tet or Chl (Fig 1).

Tab 1. Effects of tetrandrine and chlorpromazine on the collagen synthesis of human lung fibroblasts. $n=3$ wells, $\bar{x}\pm s$ (dpm). $^bP<0.05$, $^cP<0.01$ *vs* control.

Drug/μmol L^{-1}		24 h	36 h
Tet	0	606±179	1 192±231
	5.0	446±99^c	742±74^c
	10.0	372±10^c	691±35^c
	20.0	281±22^c	423±15^c
	40.0	231±8^c	256±22^c
	80.0	157±15^c	215±59^c
Chl	0	556±98	947±208
	10.0	241±22^c	427±74^c
	20.0	204±20^c	205±30^c
	40.0	184±20^c	193±22^c

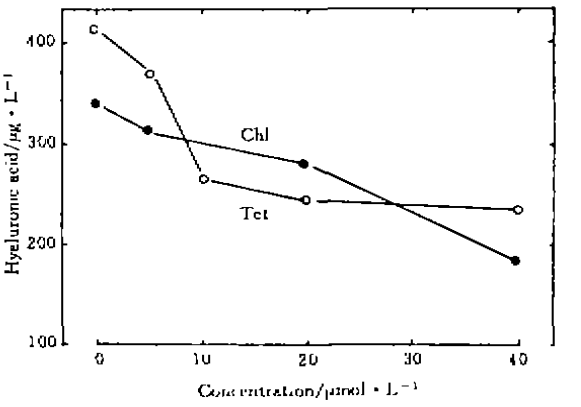

Fig 1. Effects of tetrandrine and chlorpromazine on hyaluronic acid contents in supernatants of human lung fibroblasts cultures.

DISCUSSION

In our experiment, both Tet and Chl decreased the collagen and HA production of HLF remarkably in a concentration-dependent manner. Tet can diminish cytoplasmic Ca^{2+} concentration by blocking calcium channels of the cell membrane and thereby preventing Ca^{2+} influx from extracellular fluid[3]. Otherwise, Chl reduced the activities of CaM directly. These suggested that Ca^{2+}-CaM system might participate in the regulation of collagen and HA synthesis. Tet (<300 μmol L^{-1}) could suppress the activation of CaM-

dependent cAMP diphosphatase[5]. Enhancement of intracellular cAMP levels was associated with a decrease in collagen production *in vitro*[6]. Thus, it was probably that Tet suppressed the collagen and HA synthesis by increasing intracellular cAMP.

The clinical data showed that Tet could decrease the serum content of P Ⅲ P in patients with hepatocirrhosis[7]. In our experiment, the production of collagen and HA was decreased by Tet 5－20 μmol L^{-1}, and there was no obvious toxic effect on HLF. These results suggested that Tet could be a hopeful antifibrosis drug.

REFERENCES

1 Rasmussen H. The calcium messenger system (first of two parts). N Engl J Med 1986; **314**: 1094－101.

2 Means AR, VanBerkum MFA, Bagchi I, Lu KP, Rasmussen CD. Regulatory functions of calmodulin. Pharmacol Ther 1991; **50**: 255－70.

3 Liu QY, Karpinski E, Rao MR, Pang PKT. Tetrandrine: a novel calcium channel antagonist inhibits type I calcium channels in neuroblastoma cells. Neuropharmacology 1991; **30**: 1325－31.

4 Li YR, Cai GP, Liu LF, Chen NM, Li CL. Studies on the inhibitory effect of tetrandrine on silica-induced fibrogenesis *in vitro*. Biomed Environ Sci 1988; **1**: 125－9.

5 Gao ZH, Huang SM, Wei MF. Biphasic effects of tetrandrium on the activity of calmodulin-dependent cyclic neucleotide phosphodiesterase. Mod Appl Pharm 1989; **6** (3): 1－4.

6 Berg RA, Moss J, Baum BJ, Crystal RG. Regulation of collagen production by the β-adrenergic system. J Clin Invest 1981; **67**: 1457－62.

7 Li DG, Xia WX, Lu HM, Li XH, Jiang ZM, Quan QZ, *et al*. Significance of serum procollagen-Ⅲ-peptide in reflecting the therapeutic effects of calcium-channel blockers on hepatic fibrosis. Chin J Int Med 1990; **29**: 453－6.

412-414

粉防己碱和氯丙嗪对人胚肺成纤维细胞胶原和透明质酸合成的影响

刘海林[1]，陆汉明，李定国，蒋祖明，齐 凤
（上海第二医科大学附属新华医院内科，上海200092，中国）

R965

目的：研究粉防己碱(Tet)和氯丙嗪(Chl)对人胚肺成纤维细胞胶原与透明质酸(HA)合成的影响，为应用钙拮抗剂防治器官纤维化提供依据. **方法**：采用[^{3}H]脯氨酸掺入和放射免疫法分别测定胶原与 HA 合成. **结果**：Tet 5－80 μmol L^{-1}和 Chl 10－40 μmol L^{-1}均以浓度依赖方式抑制胶原与 HA 合成. Tet 5－20 μmol L^{-1})对细胞无明显毒性却显著抑制胶原与 HA 合成($P<0.01$). **结论**：Tet 在低浓度(5－20 μmol L^{-1})时对细胞无明显毒性作用而显著抑制成纤维细胞胶原与 HA 合成，可望成为治疗器官纤维化的有效药物.

关键词 粉防己碱；氯丙嗪；胶原；透明质酸；成纤维细胞；培养的细胞